

The case for capillary rarefaction in the AKI to CKD progression: insights from multiple injury models

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24 In recent years, there has been increased appreciation regarding the potential
25 outcomes of patients following episodes of acute kidney injury. While a majority of
26 patients surviving an initial episode of AKI recover “normal” renal function, persistent
27 structural alterations following repair responses can result in development of CKD or
28 ESRD (5).

29 While clinical studies have not provided a clear understanding of the
30 mechanisms of CKD progression following AKI, animal models of injury have been used
31 to identify persistent alterations in renal structure following injury that appear to set in
32 motion progressive CKD. For example, injury-induced changes in the interstitium, such
33 as activation of myofibroblasts following renal ischemia/reperfusion, contribute to the
34 development of interstitial fibrosis while inflammation, profibrotic growth factors from
35 damaged tubules, and peritubular capillary rarefaction may amplify the fibrotic process
36 (3). With regard to rarefaction, the reduction of peritubular capillary density has been
37 proposed to augment hypoxia and fuel interstitial fibrosis (3). Interestingly, reductions in
38 peritubular capillary density have been shown in renal biopsies of patients with CKD,
39 and correlate with reduced renal survival (7).

40 However, there remains many gaps in our knowledge about the relative
41 contribution of these factors since animal studies of AKI to CKD do not routinely
42 measure renal function (i.e. GFR) directly, but rather use surrogate markers of tissue
43 injury and GFR. Alterations in factors such as plasma creatinine or the presence of
44 interstitial fibrosis may not reflect true GFR, making conclusions about progression
45 speculative. Moreover, there has been little investigation in other models of AKI outside

of ischemia reperfusion, such as those which use nephrotoxic agents with different pathophysiology.

In the current issue of *American Journal of Physiology- Renal Physiology*, Menshikh et al., publish a careful comparison of different mouse models of AKI and analyzed different features of chronic injury and progression (8). The authors used a nephrotoxic injury model using a repetitive cisplatin dosing approach and compared this with a rhabdomyolysis model induced by intramuscular injection of glycerol. These were both compared with a more widely-utilized model of unilateral I/R injury followed by delayed contralateral nephrectomy; this model has evolved to circumvent problems associated with high mortality rates in mice subjected to severe bilateral I/R (8, 9). In addition to comparing renal fibrosis and capillary density, the authors also evaluated GFR by performing sequential sinistrin clearance using a transcutaneous fluorescence technique (10). This comprehensive approach provides food for thought on the important aspects of injury which may most closely associate with progressive CKD.

Using these models, different aspects of chronic injury were observed. For example, rhabdomyolysis resulted in prominent interstitial fibrosis, but was not associated with significant decline in peritubular capillary density or GFR relative to controls. The repetitive-cisplatin model resulted in only moderate interstitial fibrosis but a significant decline in GFR. In addition, the repetitive cisplatin dosing model was associated with a prominent reduction in peritubular capillary density (8). Thus, results from two different models revealed a dissociation in capillary rarefaction and interstitial fibrosis, both of which are generally correlated with, and considered highly predictive of

kidney failure (1, 7). Moreover, the results would suggest that capillary rarefaction is most closely aligned with progression based on loss of GFR.

These observations raise a number of questions. For example, why would loss of peritubular capillary density result in a loss of glomerular filtration? As mentioned, previous hypotheses regarding peritubular capillary rarefaction invoke its potential role in hypoxia to promote interstitial fibrosis (3). However, since fibrosis is not prominent in the cisplatin model, this is not likely the explanation. It should be noted that studies from other laboratories have demonstrated an overall atrophy of renal tubules and an uncapping of glomeruli following repetitive cisplatin injection. Indeed, the degree of uncapped glomeruli was also shown to be correlated with loss of GFR (11). Whether or not the sustained tubular injury and lack of tubular repair is due to capillary rarefaction, or conversely, whether the sustained capillary rarefaction is attributable to loss of nephrons has not yet been investigated.

A second interesting aspect of the current studies relates to the comparison of these models with that of unilateral I/R and delayed nephrectomy. When short-term recovery periods of 4 weeks were examined, both fibrosis and capillary loss were associated with reduced GFR. Surprisingly, when recovery was extended to 12 weeks, the authors observed an apparent reversal of both GFR and capillary density, while renal fibrosis was persistent. This is a fascinating result that deserves additional attention as reversal of capillary density has not been prominently described. Indeed our group had initially suggested that capillary loss is permanent and vascular repair capacity is minimal following AKI (4). This may be due in part to intrinsically low levels of proliferative and angiogenic activity of renal endothelial cells (2). Recently, Dang et

91 al., suggested that FoxO1 is expressed prominently in renal EC, and that this factor
92 conveys suppressed angiogenic potential in these cells (6). Thus, if the reversal of
93 CD31+ vessel staining observed by Menshikh et al. reflects actual angiogenesis, altered
94 intracellular signaling induced by the delayed nephrectomy could allow for the activation
95 of an angiogenic response in kidney ECs which typically lack regenerative potential.
96 However, some caveats should be considered. Since only CD31 staining was used for
97 measuring capillaries, it is fair to consider whether the results represent detection of
98 dormant EC in which low CD31 expression is reactivated in response to contralateral
99 nephrectomy. This does not diminish the importance of this overall observation
100 however, as the association with vessel staining and reversal of GFR represent a
101 compelling advance in understanding the link to progression.

102 Finally, the experimental approach using different models should prompt all
103 investigators to recognize that not all models are equal. Studies such as the one
104 highlighted here provide perspective on different causes of injury resulting in various
105 long-term outcomes, which is especially important since the etiology of patients is
106 diverse. While similarities may exist, analysis of differences between models may allow
107 for more meaningful conclusions on the pathophysiology of AKI-to-CKD.

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